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New insights into developing antibiofouling surfaces for industrial photobioreactors

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Abstract

The biofouling formation of the marine microalga *N. gaditana* on non-toxic surfaces was quantified on rigid materials, both coated (with Fouling Release Coatings and nanoparticle coatings) and non-coated, to cover a wide range of surface properties from strongly hydrophobic to markedly hydrophilic under conditions similar to those prevailing in outdoor massive cultures of marine microalgae. The effect of seawater on surfaces that presented the best antibiofouling properties was also evaluated. The adhesion intensity on the different surfaces was compared with the predictions of the biocompatibility theories developed by Baier and Vogler using water adhesion tension (τ^o) as the quantitative parameter of surface wettability. For the most hydrophobic surfaces, $\tau^o \leq 0$, the microalgae adhesion density increased linearly with τ^o, following the Baier's theory trend. However, for the rest of the surfaces, $\tau^o \geq 0$, a tendency towards minimum adhesion was observed for amphiphilic surfaces with a τ^o=36 mJm^{-2}, a value close to that which minimizes cell adhesion according to Vogler's theory. The understanding and combination of the two biocompatibility theories could help to design universal antibiofouling surfaces that minimize the van der Waals forces and prevent foulant adsorption by using a thin layer of hydration.

Key words: Microalga; Photobioreactor; Anti-biofouling; Fouling Release Coatings; Biocompability theory

1. Introduction

Avoiding or delaying the appearance of biofouling in photobioreactors (PBRs) significantly lowers the costs of microalgal biomass production (Zeriouh et al., 2017a). Understanding the underlying phenomenology and fundamentals is essential for proposing solutions. Methodologies for determining the physicochemical properties of both the microalgae surface and the rigid materials that are potentially useful for fabricating PBRs used in the cultivation of planktonic marine microalgae have previously been developed (Zeriouh et al., 2017a; Zeriouh et al., 2017b; Zeriouh et al., 2019). In this context, the biocompatibility theories developed by Baier and Vogler (Vogler, 1998, 1999, 2001; Baier, 2006; Baier, 2014) may also help to design a universal antibiofouling surface for PBRs. Non-toxic hydrophobic Fouling Release Coatings (FRCs) exhibiting a critical surface tension (γ_c) value near that which minimizes cell adhesion, ca. 22-24 mJm^{-2} (hereinafter termed Baier's minimum), are currently considered to be the most promising environmentally friendly antifouling technology in the naval industry due to surface smoothness, low surface energy and a low modulus of elasticity that prompts adhered organisms to be released (Baier, 2006; Lejars et al., 2012). However, hydrophobic surfaces are also prone to protein adsorption and adhesion of certain microorganisms (mainly diatoms and bacteria) due to *hydrophobic attractions* (Yoon et al., 1997; Vogler, 2001). This has recently promoted research aimed at improving the anti-adhesion properties of Silicone-FRC coatings by increasing surface wettability using hydrophilic groups in the silicone matrix (Silicone-Amphiphilic coatings) (Galli & Martinelli, 2017; Wang & He, 2019). The amphiphilic surfaces with water adhesion tension (τ^o) values close to those that minimize cell adhesion, ca. 36 mJm^{-2} (hereinafter named Vogler's minimum) present excellent antibiofouling properties compared to the low energy surfaces (LES) located at Baier's

minimum (Seetho et al., 2015). Currently, the Silicone-FRC coatings, which incorporate amphiphilic block copolymers (mainly PEG-PDMS), are a good commercial non-toxic alternative to prevent biofouling in marine environments, especially under **dynamic conditions** (Camós-Noguer et al., 2017). These materials, both transparent and opaque, could be respectively a promising alternative for fabricating closed and open PBRs, where biofouling formation has not yet been well studied. In this work, the biofouling of the microalga *N. gaditana* was quantified on non-toxic rigid material surfaces, both coated (with FRCs and nanoparticle coatings) and non-coated, to cover a wide range of surface properties from strongly hydrophobic to markedly hydrophilic surfaces under conditions similar to those prevailing in outdoor massive microalgae cultures. The effect of natural seawater on the τ^o of surfaces that presented better antibiofouling properties was also evaluated. The adhesion intensity of the *N. gaditana* microalgae on the different surfaces was compared with the biocompatibility theory predictions developed both by Baier and by Vogler using τ^o as the quantitative parameter of surface wettability.

2. Materials and Methods

2.1 Photobioreactors and methods used to monitor the culture parameters

The microalga used was *N. gaditana* B-3. The strain was provided by the Marine Culture Collection at the Andalusian Institute of Marine Sciences (CSIC, Cádiz, Spain). The culture medium consisted of natural seawater supplemented with macro and micronutrients. The exact composition of the culture medium is described elsewhere (Zeriouh et al., 2017b). A glass bench-scale flat-panel photobioreactor (FP-PBR) was used for the indoor experiments. The operational details and the dimensions of the FP-PBR have been described elsewhere (Zeriouh et al., 2019). The FP-PBR has a 13 L

volume; it is illuminated with artificial light 12h/24h and operated in continuous mode at a dilution rate of 0.3 day^{-1}.

In contrast, the outdoor experiments were carried out in a 7.2 m^2, 900 L, pilot-scale open raceway pond (RW). The operational details and the dimensions of this RW have been previously reported (San Pedro et al., 2015). The culture circulated continuously along the photobioreactor channels at an average velocity of 35 cms^{-1}, driven by a stainless-steel paddlewheel (0.60 m in diameter). The RW was inoculated with exponential-phase inoculum grown and operated in semicontinuous mode. Filtered fresh water was added to the RW to replace the amount of evaporated water. Each week, 750 L of fresh culture medium was supplied to the RW and the same volume of culture was harvested. The experimental plan was carried out over July and August 2017. The average temperature of the culture was approximately 29.0 °C and the mean irradiance value was 2100 $\mu E\ m^{-2} \cdot s^{-1}$ (San Pedro et al., 2015).

The cell status of the cultures was checked daily by measuring the maximum photochemical yield of the photosystem II (PSII), Fv/Fm, ratio with a fluorometer (Mini-PAM-2500). The nitrate and phosphate concentrations dissolved in the supernatant and the biomass concentration in the cultures were determined spectrophotometrically as described earlier (Zeriouh et al., 2017b; Zeriouh et al., 2019). Additionally, flow cytometry was used to quantify the cultures' cell density (cells mL^{-1}). The measurements were made using a Cell Lab Quanta SC flow cytometer (Beckman Coulter Inc., Brea, CA, USA) equipped with an argon-ion excitation laser (blue light, 488 nm). At least 60,000 cells were analysed per sample. The flow rate was kept at a moderate setting (data rate = 600 events s^{-1}) to prevent interference between cells.

2.2 Preparation of both solid substrata and coatings for the adhesion test

The average surface roughness (R_a) (or arithmetic average deviation from the mean line within the assessment length) was determined using a surface profiler (Dektak 150, Veeco Instruments Inc., USA) with 1 mm scan length and a 0.111 μm/sample resolution. The R_a values for each surface are the average of several measurements (five and eight for indoor and outdoor sample surfaces, respectively) from different sites on each surface. Table 1 displays the R_a values of all the surfaces tested. A surface is considered smooth for R_a values below 0.6 μm.

In the case of the **outdoor pilot-plant experiments**, a rigid commercial polycarbonate sheet (R_a=0.014±0.004 μm) was used as the solid support for the application of the coatings. Thus, five square pieces (13cm x 13cm x 0.5cm) were washed with Alconox detergent (1%) in ultrasound for 15 min, then rinsed with abundant deionized water. The five clean square pieces were then dried at room temperature for one day. One of the square pieces served as the control (a smooth surface). Another piece was scratched with a wire brush (a Sealey WB05Y Stainless Steel Wire Brush with a Plastic Handle) to create a rough surface (R_a=1.7 μm). The other three pieces were prepared using the following commercially available coatings: (i) an antibiofouling FRC based on Silicone-Hydrogel technology (SilicOne®, Hempel, Kongens Lyngby, Denmark) (C1); (ii) a superhydrophobic and oleophobic coating that creates a surface chemistry and a texture thanks to the lotus effect caused by the nanostructure of the product's particles (Ultra-Ever Dry®, UltraTech International, Inc., Florida, USA) (C2); and (iii) a hydrophobic coating with anti-adherent properties based on nanoparticles (Plexiclean®, Nano-Care AG. Saarwellingen, Germany) (C3). The C1 coating was applied onto one of the square pieces of polycarbonate by means of bar-coating surfaces to obtain a dry, homogeneous and smooth film with an approximate thickness of 50 μm. The two other coatings, C2 and C3, were applied onto the

remaining two pieces of polycarbonate using an airbrush gun (Fengda® BD-180K, Art & Hobby, s.c.o. Praga. The Czech Republic). According to recommendations from manufacturers, during application, the air pressure was fixed at 4 bar and the coating was dispersed through a 0.25mm-diameter nozzle. The pieces coated with C1, C2 and C3 were dried at room temperature for 3 days and washed with abundant deionized water. The R_a values measured for the three resulting surfaces PC-C1, PC-C2 and PC-C3 are shown in Table 1.

For the **indoor experiments**, six rigid materials with smooth surfaces were used (Zeriouh et al., 2019): polyvinylchloride (PVC), polycarbonate (PC), polystyrene (PS), borosilicate glass (GL), stainless steel (SS) and polyethylene (PE). These materials were supplied in the form of 7.88mm-diameter disk coupons (Tyler Research Corp, Edmonton, Canada) compatible with the Modified Robbins Device (MRD) flow channel (LPMR-12PMMA, Tyler Research Corp, Edmonton, Canada). In addition, eight PC disk coupons were coated with the coatings mentioned above (C1, C2 and C3) and with Hempasil X3® (Hempel, Kongens Lyngby, Denmark) **(**C4**)**. Hempasil X3® is another non-toxic FRC that provides an invisible antibiofouling hydrogel microlayer. Coatings C1 to C4 were dispersed on the coupons using the protocol described in the second paragraph of this section.

2.3 Physicochemical properties of the materials and coatings used.

The sessile drop technique, applied with a goniometer (Drop Shape Analyzer DSA25, KRÜSS GmbH, Germany), was used to measure the contact angles on the different rigid materials and the coatings mentioned in Section 2.2. Two polar liquids (water and formamide) and another apolar liquid (diiodomethane) were employed as reference liquids. For each surface, two different samples were used. Before measuring

the contact angles, the coatings and rigid materials used in outdoor pilot-plant experiments were washed with Alconox detergent (1%), then rinsed with abundant deionized water and allowed to dry at room temperature. Meanwhile, the surfaces prepared for the indoor experiments were washed according to the protocol previously described (Zeriouh et al., 2019). Briefly, the disc coupon materials mounted in the MRD were rinsed out by closed-circuit recirculating in hot water (40 °C) containing 1% Alconox® at a shear rate of 300 s^{-1}. Subsequently, they were washed with deionized and sterilized water under the same conditions at room temperature. Next, a sodium hypochlorite (5%) solution was passed through at the same shear rate. Finally, the coupons were washed with abundant deionized and sterilized water.

The contact angles measured and the equations used for the quantification of the different surface energy components (γ_s^{LW}, γ_s^+, γ_s^-, γ_s^{AB}) for each surface, and the change in the free energy of cohesion (ΔG_{coh}) are explicitly explained in the Supplementary Material Section. The τ^o value on each surface was calculated by multiplying the water surface tension (γ_W =72.8 mJm^{-2}) by the water's contact angle cosine (θ_W) on this surface (Vogler, 1998); this being:

$$\tau^o = \gamma_W \cdot \cos\theta_W \quad [1]$$

The determination of γ_c, as defined by Zisman (1964), is valid for completely apolar liquids (Van Oss, 1993). However, Van Oss (1993) reported that, for apolar surfaces, γ_c was equal to the apolar component of the surface free energy (γ_s^{LW}) calculated using the equation developed by Van Oss et al. (1988):

$$1+\cos\theta = 2\sqrt{\gamma_S^{LW}/\gamma_L} \quad [2]$$

where θ and γ_L are the contact angle and surface tension of the apolar liquid (i.e., diiodomethane), respectively. In our work, this approach has been adopted to determine γ_c for all the different hydrophobic materials used.

2.4 Toxicity assessment of the different coatings used

Transparent 250 mL poly(propylene) cylindrical beakers, with a 70mm internal diameter, were used as culture vessels. The beakers were cleaned in an ultrasound bath for 15 minutes with detergent (Alconox, 1%), rinsed with abundant deionized water and dried at room temperature. Subsequently, the different coatings were applied to the interior of the clean beakers by means of a paint brush. Finally, the coated beakers were inoculated with a 100 mL culture volume of *N. gaditana* at a biomass concentration of 0.1 g L^{-1} d.w. in exponential growth phase, using the same culture medium as described in Section 2.1. The beakers were then placed in an orbital shaker at 100 rpm. The cultures were grown under continuous illumination at 25 °C, 12h/12h light-dark regime; this illumination being provided by 58W fluorescent lamps rendering an average irradiance on the culture vessel surface of 135 µmol photons $m^{-2}s^{-1}$. Clean beakers were used as the control (CT1) for the transparent coatings (C2 and C3). Meanwhile, the control beaker for the opaque coating **(**C1**)** was coated on the outside with an opaque black adhesive tape, available commonly in stationery stores, (CT2). In addition, a toxic matrix coating based on copper oxide (Hard racing TecCel®, Hempel) was used as the negative control. The tests were performed in duplicate. The monitoring of the biomass concentration (gL^{-1}) and the Fv/Fm in each culture was carried out using the same methodology as described in Section 2.1. Cell viability was determined by a fluorescence staining assay in diacetate (FDA) (Xiao et al., 2011). Cells stained with FDA were detected in the FL1 channel of the flow cytometer. The optimal FDA

concentration and exposure time for the culture samples in darkness was 3×10^{-3} µg mL^{-1} and 15 min, respectively.

2.5. Adhesion modules

The adhesion module for the **indoor** experiments comprised a modified Robbins device (MRD) flow channel (LPMR-12PMMA, Tyler Research Corp, Edmonton, Canada) with a 12.5mL volume and a peristaltic pump (Masterflex® L/STM Economy drive) to allow culture broth from the PBR or seawater from a tank to be pumped into the MRD. More details of the MRD can be found elsewhere (Zeriouh et al., 2019). Briefly, the MRD contained 12 evenly-spaced ports which held sample holders with recesses at the ends, into which the test disc coupons of the material or coating to be studied were inserted. Before using the MRD, it was prepared as indicated earlier (Zeriouh et al., 2019).

For the **outdoor** experiments, the adhesion assessment was performed on the five polycarbonate sheets described in Section 2.2 (PC, rugged-PC, and PC coated with C1, C2 and C3) attached to the bottom of a 900 L RW.

2.6. Measurements of the physicochemical properties of the materials and coatings exposed to natural seawater

The eight 7.88 mm-diameter polycarbonate disk coupons coated with the C1, C2, C3 and C4 (see Section 2.2) coatings, together with two other uncoated PVC coupons, were placed in the MRD holders. The adhesion module was connected to a storage tank containing 1000 L of filtered seawater (0.45µm) by means of silicone rubber tubes, and the seawater was continuously pumped through the MRD at a flow rate of 25 Lh^{-1}. The MRD output was directly connected to the drain. The drained volume (600 L) was

replaced daily. Both coated and uncoated coupons were continuously exposed to seawater for six days. On the seventh day, the adhesion module was disconnected and the coupons were disassembled from the MRD flow cell. They were then rinsed and dried at room temperature and the contact angles of the water on these surfaces were measured according to the protocol described in Section 2.3.

2.7 Adhesion tests and biofouling quantification

Indoor experiments. The six rigid and smooth materials (PVC, PC, PS, GL, SS and PE) described in Section 2.2 were placed alternately in duplicate in the MRD flow cell. After washing and sterilizing, the MRD was connected in a closed loop to the FP-PBR, which was operated in continuous operational mode at a dilution rate of D=0.3 day^{-1}. This meant that the culture concentration that circulated through the MRD was virtually the same during the entire experiment (2.5×10^8 cell mL^{-1}). The culture flow rate that circulated continuously through the MRD was 25 Lh^{-1}. In continuous culture mode, the MRD was disconnected from the FP-PBR every two days, washed gently with sterile seawater and then the density of the adhered cells per unit area (B) on each of the coupons (cells cm^{-2}) was quantified, as previously described (Zeriouh et al., 2017b; Zeriouh et al., 2019). These were then placed again in the MRD and reconnected to the FP-PBR for the next measurements.

Outdoor pilot-plant experiments. The five square pieces described above in Section 2.2 were glued to the bottom of the open pond raceway photobioreactor (RW) before being inoculated. After two months operating in semicontinuous mode, the culture was removed from the RW. To eliminate the biological material deposited by gravity on the pieces, the RW was immediately filled with filtered seawater, which was circulated through the RW channels for 24 hours at the same flow rate as that used

during the culture period (35 cm s^{-1}). Once the RW had been emptied, the square pieces were photographed to compare the intensity of the biofouling formation on their surfaces.

2.8 Statistical analyses

Statgraphics Centurion XVII (version 17.2.04) statistical software (2014, Statpoint Technologies, Inc. USA) was used for: (i) a significant difference analysis with a one-way ANOVA test (ii) a significant difference analysis with a multi-way ANOVA test.

3. Results and discussion

3.1 Physicochemical characterization of the tested surfaces

Table 1 shows the average surface roughness (Ra), the contact angles (θ), the total free surface energy components (γ_s^{TOT}), the free energy of cohesion (ΔG_{coh}) and the water adhesion tension (τ^o) of the different surfaces tested (see the calculation details in the Supplementary Materials Section). The negative variation in the free energy of cohesion ($\Delta G_{coh} < 0$) of the different uncoated polymeric materials (PVC, PS, PE, PC), the coated PC-C1, PC-C2 and PC-C3 and the stainless steel (SS) indicates that all these surfaces are hydrophobic. It should be noted that two surfaces (PC-C2 and PC-C3) are very low energy (2.4 and 11.9 mJm^{-2}) and have a very hydrophobic character compared to the rest of the surfaces. This characteristic may be due to their special surface texture, which is attributed to the nanoparticles. The glass (GL) surface is hydrophilic ($\Delta G_{coh} > 0$). The parameter τ^o, an index of water reactivity with surfaces (Vogler, 1998), is also included in Table 1. Surfaces with values of $\tau^o \leq 0$ less than zero (i.e., PC- C1, PVC, PS, and PE) can be considered **pure** hydrophobic surfaces (Vogler, 1998) (note that PC is not purely hydrophobic – although $\Delta G_{coh} < 0$, according to Vogler (Vogler, 1998) its

τ^o(=8.9 mJm^{-2})>0). For these surfaces: (i) the apolar component of the surface free energy (γ_s^{LW}) increases proportionally with τ^o and, (ii) the polar component of the surface free energy (γ_s^{AB}) is also related to τ^o although γ_s^{AB} is one order of magnitude lower than that of γ_s^{LW} for hydrophobic surfaces. However, for values of τ^o>0, γ_s^{LW} is relatively similar for all surfaces (Vogler, 1998); for example, even though the difference of τ^o between the PC and GL is 52 mJm^{-2}, the value of γ_s^{LW} only increases by 1.1 mJm^{-2} while for values of τ^o greater than zero (i.e., hydrophilic (GL) and to a lesser extent the slightly hydrophobic (PC) surfaces), the polar component increases considerably. Even though they are different concepts, γ_c and τ^o are linearly related in the (-40 mJm^{-2}≤ τ^o ≤ 25 mJm^{-2}) Vogler range (Vogler, 1998) (Fig. 1). In the same Fig. 1, the values of γ_c versus τ^o have been presented for all the surfaces used in this work. One can observe that the values of γ_c and τ^o for the polymeric surfaces including (PC-C1, PVC, PS, PE and PC) exhibit a very good linear correlation ($\gamma_c = 0.28 \cdot \tau^o + 27.85$; r^2=0.99) (Fig. 1). The hypothetical γ_c value of GL calculated from this linearity is 44.6 mJm^{-2}, a value similar to those reported in the bibliography (Baier, 1970; Dexter et al., 1975; Dexter, 1979; Meyer et al., 1988). In the case of SS (γ_c=21.7 mJm^{-2}; τ^o=-2.5 mJm^{-2}), it does not conform to the linearity observed for hydrophobic polymeric substrates. We do not have a full explanation for this but it could be due to the metallic nature of the material, perhaps the presence of electron donating groups that interact with the water's hydrogen bonds; this might slightly decrease the contact angle.

3.2 Toxicity assessment of the coatings used

For ruling out the possible toxicity of the coatings employed on the *N. gaditana* cultures, *N. gaditana* was exposed to these coatings in cylindrical beakers. The results based on the mean values of maximum photochemical yield of PSII F_v/F_M (F_r), cell

viability (V_r) and biomass yield (Cf_r), measured at the end of the batch cultures and relative to corresponding values obtained from controls, are displayed in Fig. 2. The coatings C2 and C3, based on nanoparticles, are transparent and the results were compared with the control uncoated beaker (CT1 in Fig. 2). By contrast, the opaque coating C1 was compared with the control beaker entirely covered with a darkened and non-transparent adhesive (CT2 in Fig. 2). Other opaque coating used as negative control, Hard racing TecCel® (HRT in Fig. 2), was demonstrated to be really toxic for *N. gaditana* (i.e. F_r, V_r and Cf_r equal to cero in Fig. 2). It can be appreciated from Fig. 2 that there were not statistically significant differences between the coatings C1, C2 and C3 and the corresponding controls (i.e. values of F_r, V_r and Cf_r near to 1) indicating that these coatings are not toxic for the microalgae after a long-term exposure.

3.3 Exposure of both PC-coated materials and non-coated PVC to natural sea water. Impact on the water adhesion tension (τ^o)

The Baier curve establishes an empirical relationship between γ_c and the bioadhesion strength (Baier, 2006) (see later in Fig. 5). The relevance of this curve is the establishment of a minimum biological adhesion on surfaces whose initial γ_c ranges from 20 to 30 mJm^{-2} (*theta* surfaces, or low-energy surfaces, LES). The antibiofouling efficiency of an LES increases when its modulus of elasticity decreases (E) (Lejars et al., 2012). The best fouling release surfaces are those with the lowest value of ($\sqrt{\gamma_c E}$) (Lejars et al., 2012). Currently, poly(dimethyl) siloxane elastomers (PDMS) are the best alternative for fabricating FRCs under **dynamic conditions** (Lejars et al., 2012). However, the adsorption of proteins and other foulants to these surfaces (namely *hydrophobic attraction*) are unavoidable and finally leads to the formation of a ``conditioning film´´ on the original surface (Meyer et al., 1988; Baier, 2014). The

peculiarity of some LES, when in contact with seawater, is that they preserve the γ_c value close to the initial value (22-24 mJm^{-2}) despite the formation of the conditioning film (Meyer et al., 1988) (Fig. 3A). In marine environments, the high ionic strength of seawater compresses the electrical double layer surrounding the particles (proteins, colloids, cell debris and foulants, etc); consequently, the electrostatic interactions between these particles and the submerged surfaces are negligible (Zeriouh et al., 2017a). Although the "hydrophobic attractions" (i.e., acid-base, AB, interaction as driving forces) are responsible for the formation of the "conditioning film" on the LES (Yoon et al., 1997), the absence of acceptor and donor electron groups on these surfaces (due to their hydrophobic nature) prevents the formation of covalent bonds between the conditioning film and the surface. Apparently, the union between the conditioning film and the LES is controlled by Van der Walls interactions. Regardless of the nature of the conditioning film, (γ_c) is equal to (γ_s^{LW}); therefore, the apolar component of the free energy of adhesion is practically zero (i.e., $\Delta G_{adh}^{LW} \approx 0$) (see the Supplementary Material Section). Consequently, the conditioning film was not firmly attached to the surface (and was easily detached), nor was it homogeneous; i.e., part of the original surface remains uncovered, which allows it to keep its γ_c value near the initial one (Meyer et al., 1988). Curiously, the LES used in this work (C1, C4 and PVC in Table 1), with intial γ_c between 22 and 24, retained γ_c values within the 25-29 mJm^{-2} range after 6 days natural seawater exposure (calculations can be made from the τ^o values, obtained after washing, displayed in Fig. 3B using the equation from Fig. 1 $\gamma_c = 0.28\tau^o + 27.85$). These results are in line with those reported for a LES based on a coating of dimethyl-dichlorosilane (Meyer et al., 1988) (see Fig. 3A). In addition, the three surfaces lost wettability and showed amphiphilic properties since τ^o on these surfaces increased considerably to values of 35, 23 and 38 mJm^{-2}, respectively, after natural seawater exposure (see Fig.

3B). This value of τ^o corresponds to the minimum biological interaction predicted by Vogler (amphiphilic surfaces corresponding to τ^o=30 mJm^{-2} and θ_w=65º) (Vogler, 2001). The point is discussed in detail later in Section 3.5. The partial loss of hydrophobicity after washing with the LES coatings (see Fig. 3B) indicates that (i) the surfaces slightly and permanently lose hydrophobicity after exposure to natural seawater, especially C4 and C1, and (ii) for PVC, the conditioning film does not affect γ_c although influences on the degree of hydration mean that the surfaces with the conditioning film acquire amphiphilic properties. For the coatings C1 and C4, as expected, the silicone-hydrogel technology induces these coatings to acquire an amphiphilic character after exposure to natural sea water. This response is probably due to their surface reorganization rather than the conditioning film (an important question for future studies). On the other hand, after exposure to natural seawater, the very low hydrophobic surface (PC-C2) conserves its wettability, while PC-C3, also a very hydrophobic surface, does not conserve it (see Fig. 3B). Both coatings (C2 and C3), due to the special texture of the resulting surfaces, are very low energy surfaces (2.5 and 11.9 mJm^{-2}, respectively) and are located on the left side (super hydrophobic surfaces) of the Baier curve and the left line in Vogler's theory (Type I biological response) (Vogler, 2001; Baier, 2014). It is therefore predictable that biofouling on these coatings would be greater than in LES, as can be observed in Fig. 4B.

3.4 Development of biofouling on different materials and coatings

Indoor experiments. The average adhesion density (B) of *N.gaditana* on the different materials, when the photobioreactor was operated in continuous mode (i.e., at D=0.3day^{-1} and at a steady-state biomass concentration of 2.5x10^{-8}cel mL^{-1}), is shown in Fig. 4A. The relationship between B and γ_c or τ^o of the tested materials is not

proportional. One can observe that: (i) the adhesion density is minimal on the LES (i.e.: PVC: γ_c=23.41 mJm^{-2} and SS: γ_c=21.74 mJm^{-2}) and, (ii) it seems that there could be minimum adhesion for materials with γ_c amongst those corresponding to the PC and the GL (i.e., another minimum of adhesion on the zone of amphiphilic surfaces; τ^o=35 mJm^{-2}). In decreasing order, the maximum value of B was observed on the PE (B_{max} = 1.17x10^5 cells cm^{-2}) followed by the rest of the materials: PS (41% less), GL (64% less), PC (68% less), SS (86% less) and PVC (92% less than PC).

Outdoor experiments. During the two months that these experiments lasted, the open raceway reactor was operated in semicontinuous mode (Fig. 4B). The culture maintained a healthy photosynthetic state, the mean value of Fv/Fm for the experiments was 0.604±0.034. The biomass concentration before weekly harvesting varied from 0.79-0.71 gL^{-1}. The phosphates (PO_4^{3-}) were totally consumed each week (i.e., 0.2 mM at the start of the culture, or when adding fresh culture medium, and ~0 mM just before harvesting; i.e., after 7 days), while the consumption of nitrates was about 40% each week. The nitrates concentration before weekly harvesting varied from 10.4-11.8 mM after an initial 17.7 mM at the start of the culture (or renewal). Following two months of operation, the open reactor was emptied to evaluate the degree of biofouling on the materials placed at the bottom of the RW. The coated PC-C1 showed a lower degree of biofouling compared to the other materials and coatings (Fig. 4B). After washing, the microalgal deposited on the PC-C1 surface was easily detached whereas the other materials and coatings showed almost the same degree of biofouling as that observed before these coupons were washed (Fig. 4B). The biological material deposited on PC-C1 was clearly due to the culture sedimentation after emptying the RW while the adhesion on the rest of the materials and coatings was irreversible. The photographs (Fig. 4B) also demonstrate that the material's roughness plays an important role in

biofilm development on the materials - the rugged polycarbonate (PC-rugged, R_a=1.69±0.41 µm) showed more biofouling than the smooth polycarbonate (PC-control, R_a=0.014±0.004 µm). The coated PC-C2, which is also a rugged surface, and PC-C3 did not demonstrate good antibiofouling properties. The biofilm developed on PC-C3 was like that on the PC-control piece. On the other hand, the microalgal adhesion to PC-C2 was still greater and very similar to the results obtained for the roughed-PC (note that both PC-C2 and PC-C3 are on the left side of both Baier´s curve (Baier, 2014) and the Type I biological response of Vogler´s theory (Vogler, 2001)). We do not have a coherent explanation regarding the biofouling observed with these two nanoparticle-based coatings (C2 and C3) but while the rugged surface PC-C2 continued to maintain its highly hydrophobic character throughout the experiment, PC-C3 acquired a hydrophilic character during its exposure to the marine culture medium in which the tests were carried out, yet reacquired its hydrophobic character straight after washing this surface (Fig. 3B).

3.5 How the surface biocompatibility theories of Baier and Vogler may help to develop antifouling surfaces for microalgae photobioreactors

Fig. 5 shows the two biocompatibility theories developed by Baier and Vogler that relate relative biological interaction with solid surfaces in a wide range of wettability (Vogler, 2001). Baier´s theory predicts minimum biological interaction on hydrophobic surfaces of low wettability (γ_c=22-24 mJm^{-2}, θ_w=100º-110º, τ^o=15-17 mJm^{-2}). The interaction strength increases in both directions; that is to say, when the hydrophobicity increases and also when it decreases. Vogler's theory predicts minimum adhesion on amphiphilic surfaces (τ^o=35 mJm^{-2}, θ_w=65º) (Vogler, 2001); here, it is possible to distinguish between two types of responses: (i) Type I, which corresponds to the

adsorption of proteins on hydrophobic surfaces (τ^o<35 mJm^{-2}), with adhesion intensity increasing in line with surface hydrophobicity, and (ii) Type II, which corresponds to interfacial phenomena related to the physicochemical properties of the hydration layer on hydrophilic surfaces (τ^o>35 mJm^{-2}) favouring the adsorption of polyvalent electrolytes that subsequently interact with proteins and cells – with the biological interaction density increasing in line with the surface hydrophilicity.

The adhesion density (B) on the different materials tested according to τ^o has also been included in Fig. 5. For the most hydrophobic surfaces, except for SS (i.e., PVC, PS and PE), the value of B increases linearly with τ^o in accordance with Baier's theory (r^2 = 0.99; p <0.05) up to $\tau^o \leq 0$; the minimum adhesion density was observed on the PVC surface located at Baier´s minimum (γ_c=23.4 mJm^{-2}and τ^o=-15.41 mJm^{-2}). For the $\tau^o \geq 0$ surfaces (PC and GL), the adhesion tendency could be adjusted to Vogler´s theory, which predicts a minimum of adhesion on the amphiphilic surfaces (τ^o=35 mJm^{-2}). In the same figure, we have inserted the surface coated with C1, based on FRCs-Hydrogel, which showed extraordinary antibiofouling properties. This surface, although initially having LES properties (γ_c=22 mJm^{-2} and τ^o=-21.2 mJm^{-2}), after exposure to sea water acquires amphiphilic properties, which are located just at the Vogler minimum (τ^o=35 mJm^{-2}). The PVC (located at Baier´s minimum) also acquires amphiphilic properties with a τ^o value similar to that of C1 after being in contact with sea water for 6 days (see Fig. 3B); this is probably due to the adsorption of glycoproteins, polysaccharides and other foulants present in the natural sea water (Baier, 2006), which end up forming a conditioning film that partially covers the original surface, giving it amphiphilic properties. The nature of the conditioning film is dynamic (Vogler, 1999); therefore, for long exposure times, biofouling development on chemically-induced amphiphilic surfaces should be more efficient and stable than on amphiphilic conditioning film.

The effectiveness of LES (γ_c=γ_s^{LW}=22-24 mJm^{-2}) is mainly due to the minimal Van der Waals interactions ($\gamma_s^{LW} \approx \gamma_W^{LW}$=21.8 mJm^{-2}); however, the adsorption of proteins due to *hydrophobic attractions* favours the formation of a "conditioning film". The effectiveness of amphiphilic surfaces lies in their dual character (hydrophobic-hydrophilic) - the hydrophobic part minimizes Van der Walls interactions while the polar part induces the formation of a hydration layer that reverses *hydrophobic attractions* to hydrophilic repulsion (i.e., hydration repulsion) (Van Oss, 1993). In addition, the low modulus of elasticity improves the antifouling characteristics of these surfaces. Nonetheless, microbial foulants at small length scales (e.g. microalgae, bacteria, algal spores and proteins) have such an extremely low modulus that they are incapable of deforming any reasonably robust solid substrate and thus the antibiofouling effects of chemistry-based surface design approaches dominate (Halvey et al., 2018). Therefore, consistent with our study, lowering the surface free energy should be in general sufficient to reduce biofouling at this scale. An exception is some diatom species. They possess a high modulus shell that could reach hundreds of GPa, requiring surfaces with low modulus and fine-tuned chemistry for antifouling properties (Halvey et al., 2018).

Although it has been demonstrated in this work that Hempasil X3® and SilicOne® are excellent candidates for coating raceway PBR walls, more research is needed to develop similar coatings, in terms of their physicochemical properties, but making them transparent so that they can be used in the manufacture of closed PBRs.

4. Conclusions

Rigid materials with a smooth texture, FRCs coatings based on hydrogel and coatings based on nanoparticles were used to evaluate their antibiofouling properties in

marine microalgae photobioreactors. The physicochemical properties of the original surfaces tested varied from very hydrophobic to hydrophilic. The coatings used did not alter the growth, the photosynthetic efficiency or the cell viability of the microalga *N. gaditana* even after several days of exposure. The LES (γ_c=22-24 mJm^{-2}) located at the Baier minimum, lost their wettability after exposure to natural sea water and acquired amphiphilic properties; probably due to the formation of a conditioning film, in the case of PVC, and the chemical composition of the surface, in the case of the FRCs-hydrogel (Hempasil X3® and SilicOne®) coatings. The roughness of a surface favours adhesion and the development of biofouling. The experimental data were adjusted to the biocompatibility theories developed by Baier and Vogler, where the best results were observed on LES and amphiphilic surfaces; although, after exposure to natural seawater, all these surfaces acquired an amphiphilic character.

Acknowledgements

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Conflicts of Interest Statement

The authors report no conflicts of interest

Nomenclature

Acronyms

C1	based-SilicOne® coating
C2	based-Ultra-Ever Dry® coating
C3	based- Plexiclean® coating
C4	based-Hempasil X3® coating
FDA	fluorescein diacetate
FP-PBR	laboratory-scale flat-panel photobioreactor
FRC	fouling release coating
GL	borosilicate glass
LES	low energy surfaces
MRD	modified robbins device
PBR	photobioreactor
PC	polycarbonate
PDMS	poly(dimethylsiloxane)
PE	polyethylene
PEG	polyethylene glycol
PS	polystyrene
PVC	polyvinylchloride
RW-PBR	pilot-scale race-way photobioreactor
SHT	silicone hydrogels technology
SS	stainless steel

Variables and parameters

B	microalgae adhesion intensity (cells m^{-2})
F_V	maximum variable fluorescence of chlorophyll (au)
F_M	maximum fluorescence of chlorophyll (au)
R_a	average roughness (μm)

Greek symbols

γ	surface free energy (J m^{-2})
γ_c	critical surface tension (J m^{-2})
ΔG	change in free energy (J m^{-2})
θ	contact angle, º
τ^o	water adhesion tension, J m^{-2}

Superscripts

AB	refers to acid–base, i.e. polar component
EL	refers to electrostatic component
LW	refers to Lifshitz–van der Waals, i.e. apolar or dispersive component
TOT	refers to the total sum of the all components (*AB*, *EL*, *LW*)
+	refers to electron acceptor parameter
-	refers to electron donor parameter

Subscripts

coh	refers to cohesion
D	refers to the probe liquid diiodomethane
F	refers to the probe liquid formamide
s	refers to substrate

W refers to the probe liquid water

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Legends

Fig. 1. Comparison of the predicted relationship (dashed line) between the critical surface tension (γ_c) and water adhesion tension (τ^o) reported by Vogler (1998) and the relationship (dotted line) obtained from the experimental data in this study (open and filled circles). The solid line indicates the relationship between γ_c and τ^o for only the five polymeric and hydrophobic surfaces: (SilicOne®, PVC, PC, PS and PE – filled circles).

Fig. 2. Evaluation of the toxicity of the coatings used based on the values of maximum photochemical yield of PSII (Fv/F_M), cell viability (V_r) and biomass yield (Cf_r) reached at the end of batch cultures, relative to corresponding values obtained from controls (CT1 and CT2). Data points are averages, and vertical bars are standard deviation (SD) for duplicate samples. Points without SD bars indicate that the SD was smaller than the symbol.

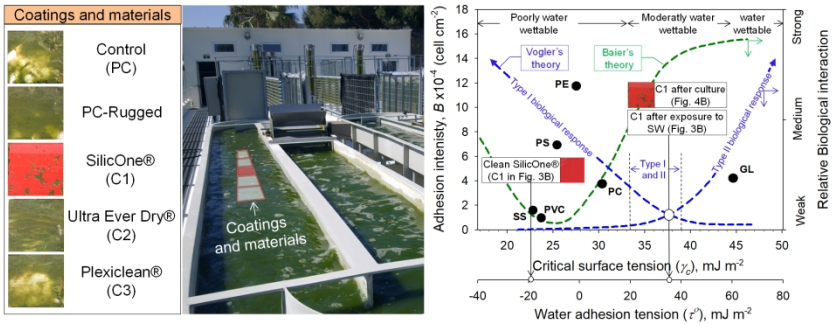
Fig. 3. Effect of seawater exposure on the water adhesion tension (τ^o) and water contact angle (θ_W) of coatings and materials used. **(A)** Temporal evolution of the critical surface tension (γ_c) of the low energy surfaces used in this study (SilicOne®, Hempasil X3® and PVC) under exposure to seawater. (empty circles and dashed line represent data reported elsewhere (Meyer et al., 1988)). **(B)** Measurements of θ_W and τ^o on PVC, PC-C2 (Ultra-Ever Dry®), PC-C3 (Plexiclean®), PC-C1 (SilicOne®) and PC-C4 (Hempasil X3®) before exposition, after 6 days of exposure to seawater and after washed with distilled water. The hatched band indicates the range of values associated with amphiphilic surfaces.

Figure 4. (A) Indoor experiments. Variation in the number of adhered cells per cm^2 (B) as a function of the critical surface tension (γ_c) and water adhesion tension (τ^o) for different materials tested in the MRD in contact with the culture of *N. gaditana*. The culture was boosted from the PBR module, operated in continuous operational mode, at a flow rate of 25 L h^{-1} providing fluid-dynamics conditions in the boundary layer of the tested materials similar to those existing in the vicinity of the wall of a tubular PBR. Lowercase letters indicate values that did not differ significantly at $p< 0.05$. **(B)** Outdoor experiments. Right: photo of a 900 L controlled raceway-photobioreactor (RW) used for the adhesion of *N.gaditana* produced in semicontinuous operational mode. Left: photos after two months of operation after harvesting all the existing microalgae culture in the RW and just after washing the RW with seawater to eliminate dirt deposited on the five samples. The values of the average surface roughness (R_a) have been included.

Figure 5. The two biocompatibility theories developed by Baier (green line) and Vogler (blue lines - reproduced from Figure 10.2 of (Vogler, 2001)). The right y-axis shows the relative strength of biological interaction, while the two x-axes show the degree of wettability of the surfaces (τ^o) and the critical surface tension (γ_c). Baier theory predicts minimum biological interaction on hydrophobic surfaces of high water contact angle ($\theta_W \approx$ 105-110º, equivalent to a $\tau^o \approx -15$ mJm^{-2} according to Vogler theory) with biological interactions increased for super-hydrophobic surfaces (left hand of the Baier curve) and for surfaces with decreased hydrophobicity (right hand of Baier curve). On the other hand, Vogler theory predicts minimum biological interaction on amphiphilic surfaces ($\theta_W \approx$ 65º; $\tau^o \approx$ 35-38 mJm^{-2}). Biological interaction increases as either the hydrophobicity (type I biological response) or the hydrophilicity increases (type II).

Black circles indicate the number of cells adhered on the different surface materials tested in the indoor experiments (left *y*-axis) as a function of their physico-chemical surface properties, τ^o and γ_c,(from Figure 4A). In addition, inserts indicate the values of γ_c and τ^o for PC-C1, SilicOne®, which showed the best antibiofouling properties in the outdoor pilot plant experiments, before (hydrophobic) and after (amphiphilic) exposure to natural seawater (from Figure 3B).

GRAPHICAL ABSTRACT



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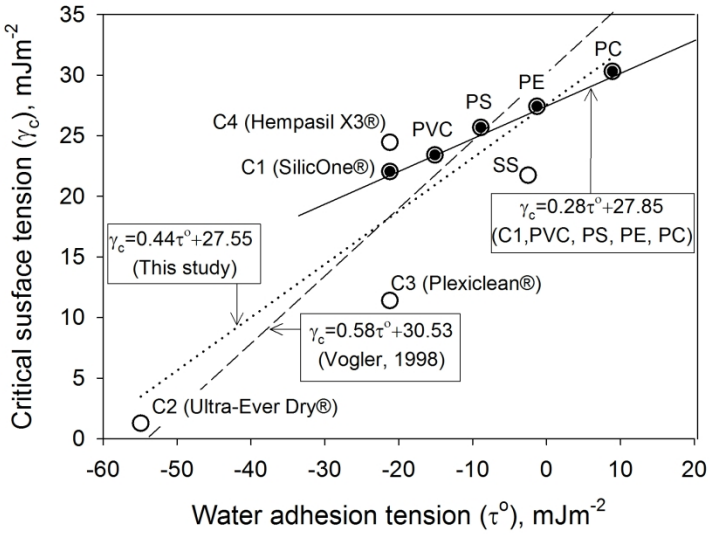

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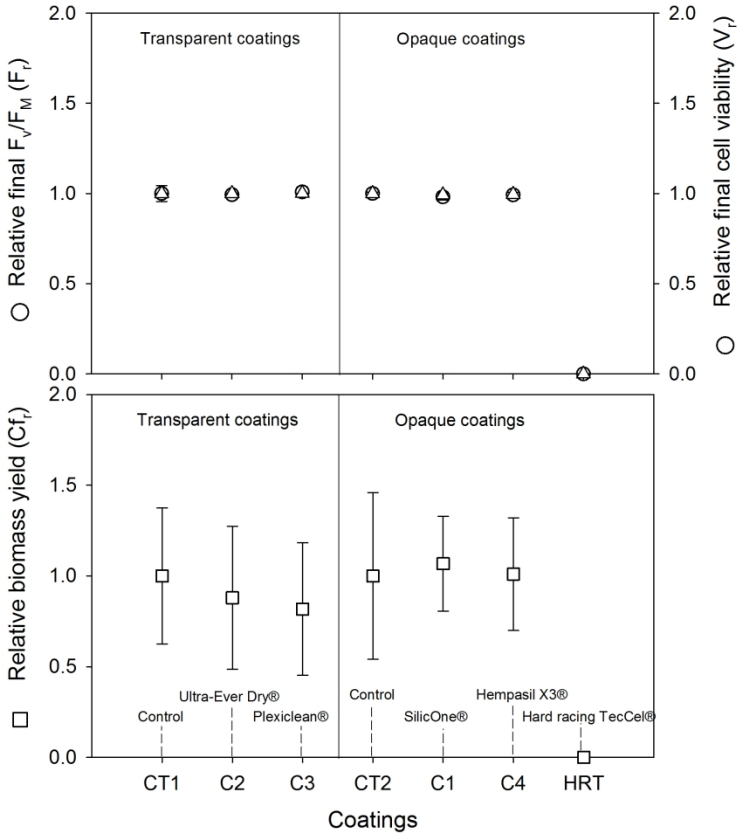

Figure 2

Evaluation of the toxicity of the coatings used based on the values of maximum photochemical yield of PSII (F_V/F_M), cell viability (V_r) and biomass yield (Cf_r) reached at the end of batch cultures, relative to corresponding values obtained from controls (CT1 and CT2). Data points are averages, and vertical bars are standard deviation (SD) for duplicate samples. Points without SD bars indicate that the SD was smaller than the symbol.

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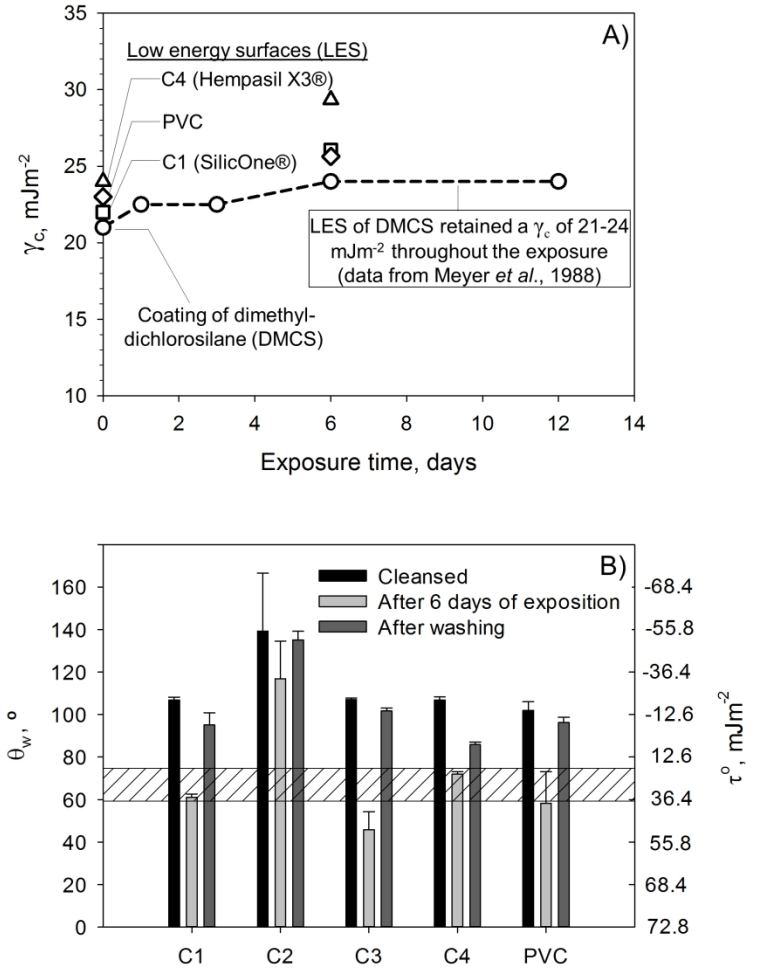

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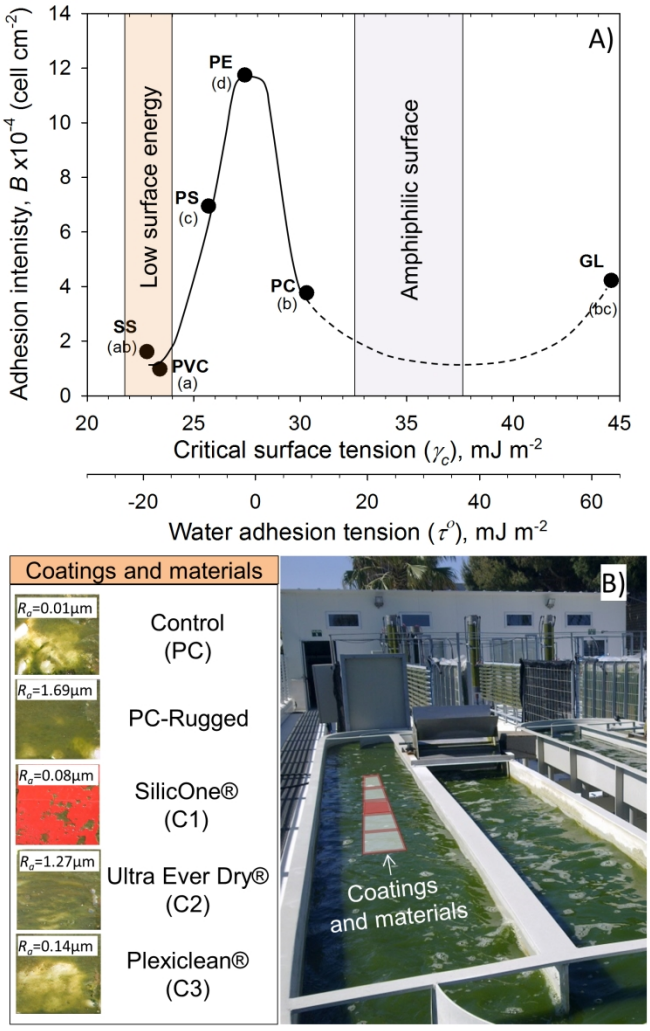

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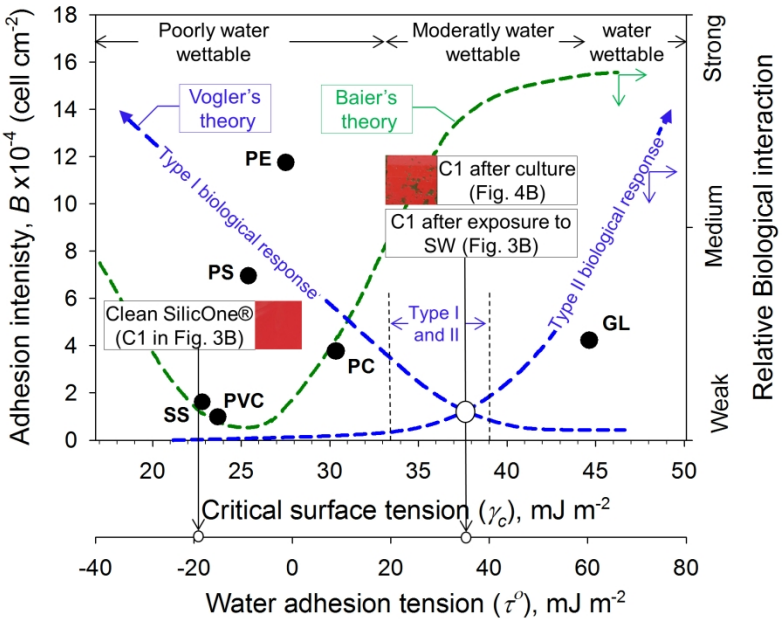

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pilot plant experiments, before (hydrophobic) and after (amphiphilic) exposure to natural seawater (from Figure 3B).

190x254mm (300 x 300 DPI)

Table 1. Surface roughness, contact angles, total free surface energy components, critical surface tension, free energy of cohesion and water adhesion tension of the different substrates

	R_a, µm	Contact angles, θ (°)			Surface energy components, free energy of cohesion and water adhesion tension (mJm^{-2})						
		θ_W	θ_F	θ_D	γ_s^{LW}	γ_s^+	γ_s^-	γ_s^{AB}	γ_s^{TOT}	ΔG_{coh}	τ^o
PC	0.03±0.00	83±2	67±1	57±4	30.30	0.10	6.84	2.98	31.96	-47.45	8.9
PVC	0.47±0.02	102±3	83±3	69±3	23.41	0.01	1.30	0.23	23.64	-77.46	-15.1
PS	0.03±0.00	97±2	77±0	65±1	25.68	0.83	1.83	2.45	28.15	-61.52	-8.9
PE	0.13±0.01	91±3	77±2	62±2	27.42	0.01	5.56	0.47	27.89	-53.94	-1.3
SS	0.34±0.04	92±1	80±5	72±2	21.74	0.04	6.01	0.98	22.72	-50.40	-2.5
GL	0.13±0.01	34±1	58±5	55±2	31.45	0.33	73.37	9.84	41.29	61.18	60.4
PC-C1 (SilicOne®)	0.08±0.01	107±1	90±1	72±3	22.04	0.09	1.18	0.66	22.70	-75.23	-21.2
PC-C2 (Ultra-Ever Dry®)	1.27±0.33	139±22	143±1	133±2	1.28	0.24	1.46	1.17	2.45	-95.20	-54.9
PC-C3 (Plexiclean®)	0.14±0.05	107±13	101±13	93±3	11.40	0.02	4.44	0.53	11.93	-61.30	-21.2
PC-C4 (Hempasil X3®)	0.31±0.02	107±1	88±2	67±7	24.45	0.13	0.85	0.67	25.13	-77.53	-21.2

(R_a): average surface roughness:(θ): the measured contact angle; (γ): surface free energy; (ΔG): change in free energy; (τ^o): water adhesion tension. The subscripts: *W*, *F*, *D*, *s* and *coh* refer the water, formamide, diiodomethane, susbstrata and cohesion, respectively. The superscripts: *LW*, +, -, *AB* and *TOT* refer the Lifshitz–van der Waals component, electron acceptor parameter, electron donor parameter, acid–base component and total sum of the all components (*AB*, *EL*), respectively.

Supplementary Material

1. *Determination of the physicochemical properties of the surfaces tested*

The results from contact angle measurements and known surface tension properties of three probe liquids (W: water, D: diiodomethane, F: Formamide) are used to calculate the surface energy parameters of the substrata based on the extended Young's equation according to Eq [1]:

$$\cos(\theta_i) = -1 + \frac{2\cdot(\gamma_s^{LW}\cdot\gamma_i^{LW})^{0.5}}{\gamma_i} + \frac{2\cdot(\gamma_s^{+}\cdot\gamma_i^{-})^{0.5}}{\gamma_i} + \frac{2\cdot(\gamma_s^{-}\cdot\gamma_i^{+})^{0.5}}{\gamma_i} \quad [1]$$

The subscript i refer the probe liquid and s refer the substrata; θ: The measured contact angle; (γ^{LW}): The apolar (LW) Surface energy component; (γ^{+},γ^{-}): The electron donor and acceptor parameters, respectively. The values of the components of the surface tension of the three test liquids used are:

$$\gamma_D = 50.8\frac{mJ}{m^2};\quad \gamma_D^{+} = 0\frac{mJ}{m^2};\quad \gamma_D^{-} = 0\frac{mJ}{m^2};\quad \gamma_D^{LW} = 50.8\frac{mJ}{m^2}$$

$$\gamma_F = 58\frac{mJ}{m^2};\quad \gamma_F^{+} = 2.28\frac{mJ}{m^2};\quad \gamma_F^{-} = 39.6\frac{mJ}{m^2};\quad \gamma_F^{LW} = 39\frac{mJ}{m^2}$$

$$\gamma_W = 72.8\frac{mJ}{m^2};\quad \gamma_W^{+} = 25.5\frac{mJ}{m^2};\quad \gamma_W^{-} = 25.5\frac{mJ}{m^2};\quad \gamma_W^{LW} = 21.8\frac{mJ}{m^2}$$

The contact angles measured with the diiodomethane are used to solve apolar component (γ_s^{LW}) of the surface energy (Eq. [1]), and, the contact angles measured with the other two probe liquids, water and formamide, are used to solve for the other two unknown surface energy parameters, (γ_s^{+}) and (γ_s^{-}) (Eq. [1]). Polar surface energy component (γ_s^{AB}) of the surface is calculated based on the calculated (γ_s^{+}) and (γ_s^{-}) (Eq. [2]). The total surface energy (γ_s^{TOT}) is calculated based on (γ_s^{AB}) and (γ_s^{LW}) (Eq. [3]).

$$\gamma_s^{AB} = 2\cdot\sqrt{\gamma_s^{+}\cdot\gamma_s^{-}} \quad [2]$$

$$\gamma_s = \gamma_s^{AB} + \gamma_s^{LW} \quad [3]$$

2. *Degree of hydrophobicity and hydrophilicity of the surfaces tested*

The hydrophobicity and hydrophilicity of surfaces are determined based on the free energy of cohesion ΔG_{coh} according to Eq. [4],

$$\Delta G_{coh} = -2 \cdot (\sqrt{\gamma_s^{LW}} - \sqrt{\gamma_W^{LW}})^2 - [4(\sqrt{\gamma_s^+ \cdot \gamma_s^-} + \sqrt{\gamma_W^+ \cdot \gamma_W^-} - \sqrt{\gamma_s^+ \cdot \gamma_W^-} - \sqrt{\gamma_s^- \cdot \gamma_W^+})] \quad [4]$$

While a negative ΔG_{coh} indicates hydrophobicity, a positive value indicates hydrophilicity.

3. *Determination of the Van Der Waals component of the change in free energy of adhesion*

The LW component of surface free energy of particles (γ_p^{LW}), aqueous medium (γ_W^{LW}) and substrate (γ_s^{LW}) are taken into account to calculate LW component of the change in free energy of adhesion (Eq.[5]).

$$\Delta G_{adh}^{LW} = -2 \cdot (\sqrt{\gamma_p^{LW}} - \sqrt{\gamma_W^{LW}}) \cdot (\sqrt{\gamma_s^{LW}} - \sqrt{\gamma_W^{LW}}) \quad [5]$$

While a negative ΔG_{adh}^{LW} indicates adhesion, a positive value indicates repulsion between the substrata and particle.